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Regulation of Jadomycin B Production in *Streptomyces venezuelae* ISP5230: Involvement of a Repressor Gene, *jadR*$_2$

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The nucleotide sequence of a region upstream of the type II polyketide synthase genes in the cluster for biosynthesis of the polyketide antibiotic jadomycin B in *Streptomyces venezuelae* contained an open reading frame encoding a sequence of 196 amino acids that resembled sequences deduced for a group of repressor proteins. The strongest similarity was to EnvR of *Escherichia coli*, but the sequence also resembled MtrR, AcrR, TetC, and TcmR, all of which are involved in regulating resistance to antibiotics or toxic hydrophobic substances in the environment. Disruption of the nucleotide sequence of this putative *S. venezuelae* repressor gene (*jadR*$_2$), by insertion of an apramycin resistance gene at an internal *Mlu*I site, and replacement of the chromosomal gene generated mutants that produced jadomycin B without the stress treatments (exposure to heat shock or to toxic concentrations of ethanol) required for jadomycin B production by the wild type. When cultures of the disruption mutants were ethanol stressed, they overproduced the antibiotic. From these results it was concluded that expression of the jadomycin B biosynthesis genes are negatively regulated by *jadR*$_2$.

Antibiotic production in streptomycetes appears to be regulated in response to nutritional status and a variety of environmental conditions, many of which have an effect on growth rate (3–5, 10). Investigation of the regulatory mechanisms should provide insight into the processes of metabolic and morphological development in streptomycetes and is of potential value for manipulating industrially important strains of these gram-positive soil bacteria, such as those used in antibiotic production. From recent studies, evidence for a regulatory hierarchy encompassing sporulation and antibiotic production has begun to emerge (21). There are strong indications that pathway-specific regulatory genes genetically linked to biosynthetic gene clusters activate transcription of the genes for antibiotic biosynthesis. The activity of the pathway-specific genes is in turn determined by genes of higher rank in the regulatory hierarchy, including global regulatory genes responsive to the environment (10).

While the role of positive regulation has been supported by the characterization of a number of pathway-specific activator genes (15, 26, 39, 40), reports of negative regulation as a factor in the control of antibiotic production are less common. The presence of a repressor gene that regulates methylenomycin production in *Streptomyces coelicolor* A3(2) was deduced from the increased output of the antibiotic that followed some prophage integrations at one end of the biosynthesis gene cluster (13); sequence analysis of the gene (*mmyR*) disrupted by the prophage showed that its deduced product resembles repressors of the TetR family (4). Other genes encoding proteins with sequence similarity to known repressors have been identified within the biosynthetic clusters for actinorhodin (12), tetracenomycin C (16), and daunorubicin (29). However, the actinorhodin and tetracenomycin genes each regulate adjacent resistance genes, rather than biosynthetic genes, and the function of the repressor gene (*dnrO*) at the *dnrR*$_2$ locus in the daunorubicin producer *Streptomyces peucetius* has not yet been elucidated.

Streptomyces venezuelae ISP5230 produces two antibiotics, chloramphenicol and the novel anguacycline antibiotic jadomycin B (1a). These antibiotics are produced under distinctly different conditions; chloramphenicol biosynthesis is a response to moderate nutrient limitation, whereas jadomycin B is formed only when cultures grown in a medium containing poorly assimilated sources of carbon and nitrogen are exposed to additional stress, such as heat shock, phage infection, or exposure to toxic concentrations of ethanol (7, 8). The regulatory mechanism imposing a requirement for severe environmental stress to initiate jadomycin B biosynthesis appears to be pathway specific, since it does not elicit concomitant production of chloramphenicol. Although the biosynthesis of jadomycin B has not been examined in detail, the inference from inspection of the chemical structure that it is derived from a polyketide intermediate has been supported by evidence that the molecule is enriched at appropriate positions by incorporation of isotopically labeled acetate (1). In addition, a cluster of genes involved in the biosynthesis of jadomycin B has recently been cloned and has been shown by nucleotide sequence analysis to bear a close relationship to known type II polyketide synthase gene clusters (19). In the present paper, we present evidence that formation of the antibiotic is negatively regulated by the product of *jadR*$_2$, a repressor gene located upstream of the polyketide biosynthesis genes.

MATERIALS AND METHODS

Bacterial strains, plasmids, phages, and culture conditions. The bacteria, plasmids, and phages used are listed in Table 1. *Escherichia coli* and *S. venezuelae* cultures were grown as described previously (19).

Transformation procedures. Competent *E. coli* cells were prepared and transformed with DNA as described by Hopwood et al. (20). Protoplasts of *S. venezuelae* were prepared and transformed by modifying the methods of Hopwood et al. (20) as follows: MYME medium, containing 10.3% sucrose, 1% maltose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, 5 mM $MgCl_2$, and 1% glycine, was inoculated with *S. venezuelae* spores and incubated for 48 h at 27°C; protoplasts were prepared from the mycelium in modified P buffer, containing 7.32% mannitol, 70 mM NaCl, 10 mM $MgCl_2$, 20 mM $CaCl_2$, and 25 mM TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] at pH 7.2; the protoplast regeneration medium contained 7.32% mannitol and 1% maltose in place

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TABLE 1. Bacterial strains, phages, plasmids and cosmid

Strain, phage, plasmid, or cosmid	Phenotype, description, and/or construction	Source or reference
S. venezuelae strains		
ISP5230	Wild type	38
VS661a and VS661b	Contain pJV84 integrated by single crossover	This work
VS662a and VS662b	Mutants carrying Am^r after $jadR_2$ replacement	This work
Phages		
Lambda 8	Lambda GEM-11 with a 10.3-kb insert containing part of the *jad* cluster from *S. venezuelae* ISP5230	18
Lambda LH7	Lambda GEM-11 with a 17.8-kb insert containing part of the *jad* gene cluster of *S. venezuelae* ISP5230	18
Plasmids or cosmid		
pBluescript II SK(+) and SK(−)	Phagemid vectors (Ap^r *lacZ′*) with oppositely oriented polylinkers	Stratagene
pHJL400	*Streptomyces-E. coli* bifunctional vector (*tsr* Ap^r *lacZ′*)	24
pJV70A	pBluescript II SK(+) with 4.3-kb *Sac*I insert from phage LH7	This work
pJV70B	Same as pJV70A but insert cloned in opposite orientation	This work
pJV71A	pBluescript II KS(+) with 3.9-kb *Kpn*I insert from pJV70A	This work
pJV71B	Same as pJV71A but insert cloned in opposite orientation	This work
pJV72	pHJL400B with 4.3-kb *Sac*I insert from pJV70A	This work
pJV80	pHJL400 with 3.3-kb *Hin*dIII insert from pJV72	This work
pKC462a	Cosmid containing Am^r	36
pJV84	pJV80 containing Am^r in a 1.5-kb fragment originally excised from pKC42a with *Bam*HI-*Pst*I, recloned in pBluescript II SK(+), excised with *Hin*dIII-*Bam*HI, blunt ended, and recloned in the blunted *Mlu*I site of the vector	This work

of sucrose and glucose, respectively; and T buffer contained 25% polyethylene glycol 4000 (Sigma) in P buffer. Transformants were selected by overlaying the agar surface with soft nutrient agar containing thiostrepton to give a final concentration in the agar of 25 μg/ml (20).

DNA manipulations. Plasmid DNA was isolated by the standard procedure (32) or by the alkaline lysis method of Kieser (22). Genomic DNA from *S. venezuelae* was isolated by the method of Hopwood et al. (20). Restriction enzymes, T4 DNA ligase, and the Klenow fragment of DNA polymerase I were used according to the suppliers' instructions.

Nucleotide sequencing and sequence analysis. DNA fragments were subcloned in pBluescript II SK(+) and KS(+). Overlapping deletion clones were generated by the exonuclease III procedure, and templates from both DNA strands were obtained from phagemid transformants of *E. coli* TG1 (32); they were sequenced by the dideoxynucleotide method (33) with Sequenase version 2.0 (Amersham) and α-^{35}S-dATP. Regions of sequence uncertainty were resequenced with 7-deaza-dGTP and dITP replacing dGTP in the sequencing reactions. The sequence was analyzed with version 7.0 software developed by the Genetics Computer Group, Madison, Wis.

Hybridization. Restriction enzyme digests of DNA samples were fractionated by electrophoresis in 0.8% agarose, transferred to a nylon membrane, and hybridized as described by Southern (35). The DNA probe was labeled with digoxigenin-dCTP by the random priming procedure. Hybridization was carried out in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0)–2% (wt/vol) Hammarsten casein (as a blocking reagent)–0.1% (wt/vol) *N*-lauroylsarcosine–0.02% (wt/vol) sodium dodecyl sulfate (SDS) at 65°C. The membrane was washed at a high stringency (0.1× SSC and 0.1% SDS at 65°C). Signals were detected by the chemiluminescence procedure (Boehringer Mannheim).

Assays for jadomycin production. The general procedure for assay of jadomycin production was as described previously (19). Jadomycin B production was assayed at intervals during the growth at 27°C of shaken cultures (50 ml/250-ml Erlenmeyer flask) in galactose-isoleucine medium (8). Cultures were initiated from 10% (vol/vol) vegetative inocula prepared by incubating spores from the relevant *S. venezuelae* strains in MYM medium (38) for 24 h under the conditions used for jadomycin B production. At 6 h after inoculation, cultures were supplemented when needed with ethanol (6%, vol/vol) and incubated for a further 48 h. Jadomycin B production was measured by withdrawing 5-ml samples and using high-performance liquid chromatography to analyze the material extracted with ethyl acetate from the filtered broth (19).

Nucleotide sequence accession number. The DNA sequence data reported here have been deposited in GenBank under accession number U24659.

RESULTS

Nucleotide sequence of the region containing $jadR_2$. The 19.8-kb region of *S. venezuelae* ISP5230 DNA cloned as overlapping fragments in recombinant lambda phages 8 and LH7 (Fig. 1) (18) contains a series of open reading frames (ORFs) constituting a polyketide synthase gene cluster (19). The 4.3-kb *Sac*I-*Sac*I segment lying upstream of these genes and separated from them by 1.35 kb of DNA was subcloned in both orientations in the phagemid vector pBluescript II SK(+) to give pJV70A/B. To facilitate the generation of deletion clones for sequencing this segment, an internal 3.9-kb *Kpn*I-*Kpn*I fragment was subcloned in both orientations in pBluescript II KS(+) to give pJV71A/B. The sequences of the pJV71A/B inserts and of the outermost *Sac*I-*Kpn*I regions of the pJV70A/B inserts were determined and were analyzed with the CODON PREFERENCE program (6). The analysis indicated (Fig. 1) that the 4.3-kb *Sac*I-*Sac*I fragment contained one partial and three complete ORFs; each ORF exhibited the biased codon usage characteristic of coding DNA in the G+C-rich genomes of streptomycetes (43). The analysis also showed that the ORF located between the *Dra*I and *Dde*I restriction sites (designated $jadR_2$ in Fig. 1) was oriented oppositely from the other three ORFs and from the nearby polyketide synthase genes.

The nucleotide sequence of $jadR_2$ is shown in Fig. 2. The most likely start codon is the GTG at nucleotides (nt) 500 to 502, which is preceded 6 nt upstream by a plausible ribosome binding site (GGAG; ΔG, −9.4 kcal [ca. −39 kJ] per mol [41]). In the 647-nt region upstream of $jadR_2$, between the start codons for $jadR_2$ and the oppositely oriented ORF designated $jadR_1$ in Fig. 1, the sequence is A+T rich. Its average G+C content is 56%, compared with an average overall G+C content in streptomycete genomes of 74% (14); thus, the A+T content of this region exceeds even that of streptomycete rRNA genes (57 to 60% G+C [43]). Sequences matching the −10 and −35 consensus hexamers for typical vegetative promoters are present in the region but lack the correct spacing for RNA polymerase recognition (27, 37). The first in-frame stop codon in the $jadR_2$ ORF is a TGA at nt 1088 to 1090; thus, the nucleotide sequence is expected to encode a polypeptide of 196 amino acids, with an M_r of 21,156 and an estimated isoelectric point of 7.85. The intergenic region downstream of $jadR_2$ is relatively short (105 nt), but the sequence contains imperfect inverted repeats that could generate stem-loop

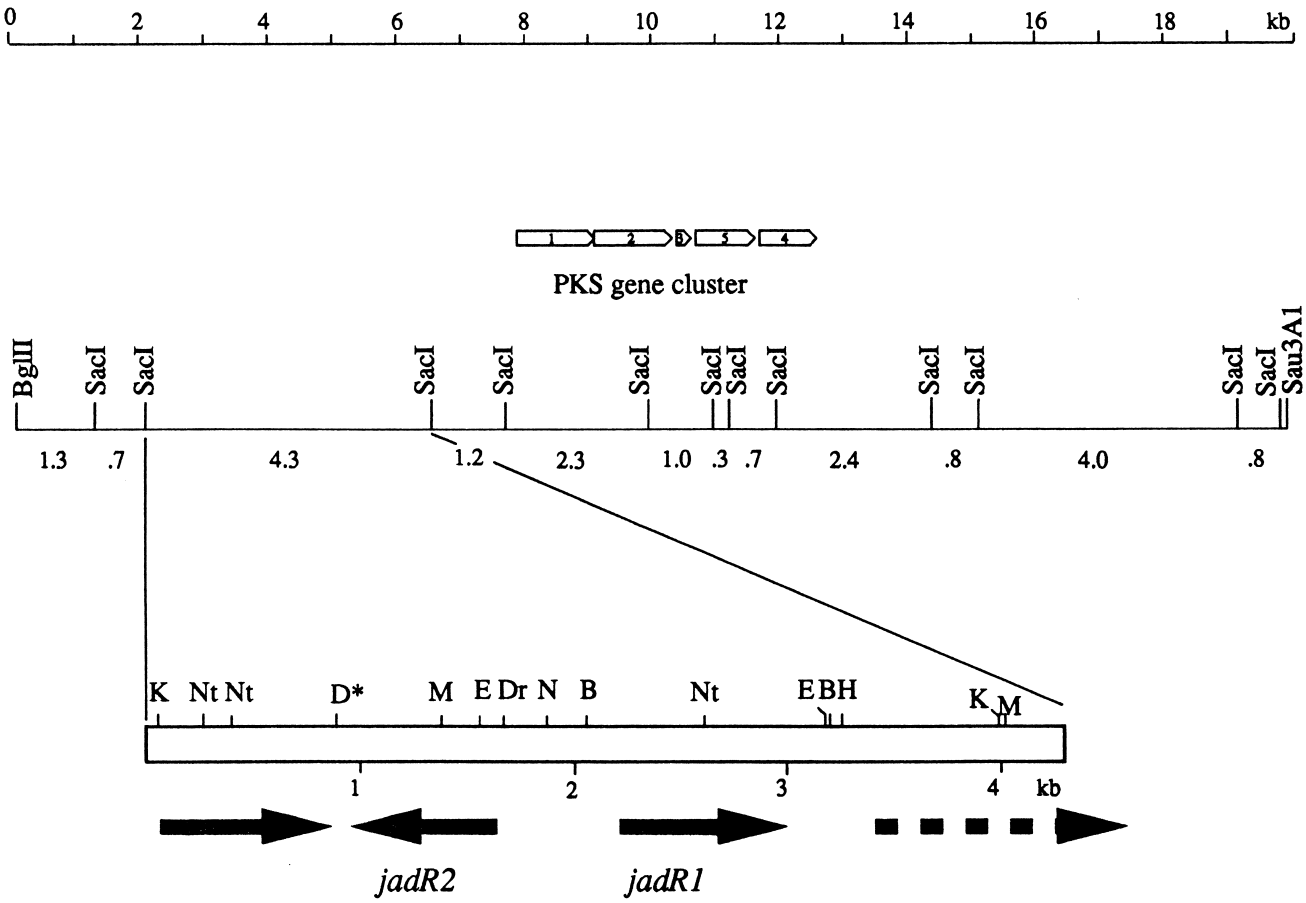

FIG. 1. *Sac*I restriction map of the 19.8-kb fragment of *S. venezuelae* ISP5230 genomic DNA cloned as overlapping inserts in lambda 8 and lambda LH7. The location of a polyketide synthase (PKS) gene cluster described by Han et al. (19) is shown. The expansion shows a detailed restriction map of the 4.3-kb segment subcloned in pBluescript II and sequenced. Arrows indicate the ORFs and their orientations. The broken arrow shows the position and orientation of the incomplete ORF. Abbreviations: B, *Bam*HI; E, *Eco*RI; D, *Dde*I; Dr, *Dra*I; H, *Hin*dIII; K, *Kpn*I; M, *Mlu*I; N, *Nco*I; Nt, *Not*I. The asterisk indicates that the site is not unique.

structures functioning as transcriptional terminators for $jadR_2$ or the convergent downstream ORF, or both.

Characteristics of the deduced $jadR_2$ product. A comparison of the amino acid sequence deduced for $jadR_2$ with sequences in protein databases showed that the closest resemblance (42% identity and 68% similarity over 47 amino acids) was to EnvR of *E. coli* (23). $JadR_2$ also resembled, in decreasing order, the products of *mtrR* from gonococci (30), *acrR* from *E. coli* (44) (cited in reference 30), *tetC* of Tn*10* from *E. coli* (34), and *tcmR* from *Streptomyces glaucescens* (16). Similarities in the deduced amino acid sequences of these proteins are concentrated within the region nearest the N terminus (Fig. 3); beyond approximately 60 residues from the N termini, the molecules bear little resemblance to one another. The N-terminal regions are predicted to form helix-turn-helix structures capable of binding to DNA target sequences (42). On the basis of the location of a helix-turn-helix structure predicted to be in $jadR_2$ by the method of Brennan and Mathews (2), the DNA-binding region of the repressor lies between residues 31 and 52 (Fig. 3).

Disruption of $jadR_2$ in the *S. venezuelae* chromosome. To introduce a mutation into $jadR_2$, a region of DNA containing the gene was excised as a 3.2-kb *Sac*I-*Hin*dIII fragment from pJV70 and subcloned as pJV80 in the bifunctional *Streptomyces-E. coli* vector pHJL400 (24). Plasmid pJV80 was linearized with *Mlu*I, and the ends were blunted by treatment with the Klenow fragment of DNA polymerase I. The DNA was then ligated to a 1.5-kb fragment containing the apramycin resistance gene from pKC462a (36), which had been subcloned in pBluescript II SK(+), and then excised from this vector and blunt ended. The replacement vector thus constructed (pJV84; Fig. 4A) was determined by digestion with *Bam*HI to contain the apramycin resistance gene in the same orientation for transcription as the interrupted $jadR_2$. To evade restriction systems causing low rates of transformation, the plasmid was propagated in and reextracted from the DNA methylation-deficient *E. coli* strain ET12567 (25) before use in *S. venezuelae* ISP5230.

Genomic DNA samples from transformants (VS661) selected for resistance to both thiostrepton (25 μg/ml) and apramycin (50 μg/ml) were screened for hybridization with pHJL400 to determine whether they contained pJV84 DNA. Strains in which chromosomal but not free plasmid DNA hybridized to the probe were allowed to sporulate, and the spores were plated on MYM agar containing thiostrepton (10 μg/ml) or apramycin (50 μg/ml) to determine their resistance to these antibiotics. More than 80% of the VS661 spores resistant to apramycin were sensitive to thiostrepton. Several of the Am^r $Thio^s$ colonies (VS662), which were anticipated to have undergone gene replacement by a second crossover event, were retained for further examination. Genomic DNA from two isolates each of strains VS661 and VS662 was digested with

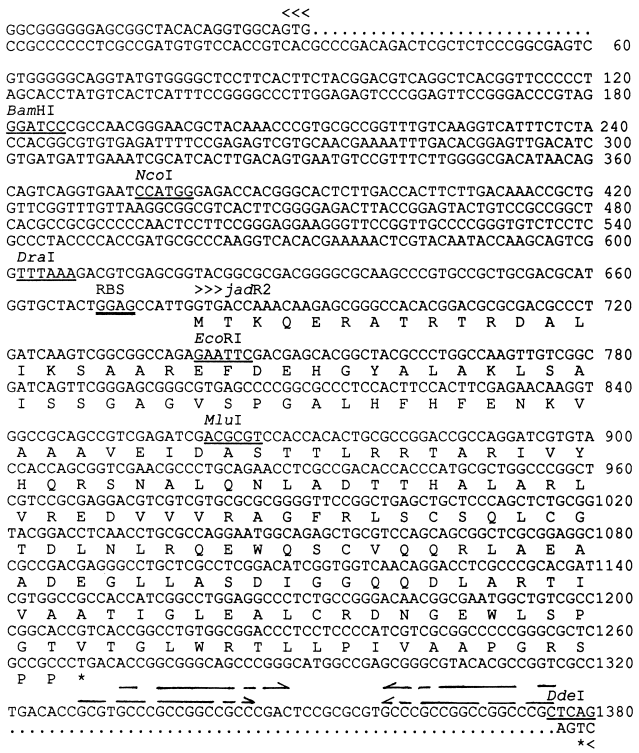

FIG. 2. Nucleotide and deduced amino acid sequences of a 1,380-bp segment of DNA containing *jadR*$_2$. Restriction sites are underlined. Putative start codons are shown initiating the translated sequences, and an asterisk indicates the stop codon. A plausible ribosome binding site (RBS) for *jadR*$_2$ is double underlined. Arrows identify imperfect inverted repeats.

*Sac*I and examined by Southern hybridization with pJV84 as the probe (Fig. 4B). As expected, DNA from the wild-type strain gave a signal corresponding to a 4.3-kb fragment. The probe hybridized with 0.8-, 1.8-, 3.2-, and 9.1-kb fragments in DNA from one VS661 strain (VS661a) but with an additional 8.0-kb fragment in DNA from a second strain (VS661b). The absence of a signal at 4.3 kb in the *Sac*I digests of VS661a and VS661b indicated that neither strain contained the parental *jadR*$_2$ without an apramycin gene insert. The signals at 3.2 and 9.1 kb in both strains indicated that pJV84 had integrated into the *S. venezuelae* chromosome by homologous recombination at the location designated x in Fig. 4A. The absence of the 8.0-kb signal in VS661a indicated that this strain contained a single copy of pJV84; its presence in VS661b showed that here two or more copies of pJV84 had integrated in tandem. An alternative explanation, that the 8.0-kb signal arose from free pJV84, is excluded by the absence of free plasmid in VS661 established during the initial screening of transformants for hybridization (see above). Tandem plasmid integrations have been observed previously in *S. venezuelae* (19, 31). In all VS662 strains, *Sac*I digests probed with pJV84 gave hybridizing signals at only 0.8, 1.8, and 3.2 kb. Fragments of this size are consistent with replacement of *jadR*$_2$ by the disrupted gene through a crossover at position y in Fig. 4A. In genomic DNA from VS661 strains, the greater intensity of the hybridization signals at 0.8, 1.8, and 3.2 kb than of those at 8.0 and 9.1 kb implies that the cultures contained double- as well as single-crossover mutants.

Analysis of *jadR*$_2$ mutant phenotypes. Jadomycin B production by the *jadR*$_2$ mutants and the wild type was compared in liquid cultures grown under normal nutritional stress, as well as in cultures grown with an ethanol supplement to give severely stressed conditions. In the nutrient-poor galactose-isoleucine medium, in which all of the strains grew slowly, wild-type cultures were unpigmented and did not produce jadomycin B; each of the VS661 and VS662 strains was similarly pigmented and accumulated 25 (±5) μg of jadomycin B per ml (Fig. 5). Supplementation of cultures with 6% (vol/vol) ethanol after 6 h of growth in this medium allowed the wild-type strain to produce 58 μg of jadomycin B per ml; strains VS661 and VS662 under these conditions each responded similarly and produced 82 (±10) μg/ml. To determine whether the similar production was due to a high rate of homologous recombination in VS661 strains, giving rise to a large proportion of VS662 in cultures, the effect of adding thiostrepton (10 μg/ml) to select against VS662 was examined. The thiostrepton supplement did not alter jadomycin production in VS661 cultures, indicating that the increased level resulted from integration of the plasmid by a single crossover.

DISCUSSION

The requirement for environmental stress superimposed on a nutritional imbalance to elicit jadomycin B synthesis in wild-type *S. venezuelae* cultures implies the existence of a control mechanism different from those investigated for streptomycetes in which antibiotics are produced as a result of a physiological imbalance alone (9, 10). The additional need for environmental stress is explained if genes for producing the antibiotic continue to be repressed under conditions that activate pathway-specific regulatory genes in other systems [e.g., those for the biosynthesis of actinorhodin or undecylprodigiosin in *S. coelicolor* A3(2) (21)]. Similarities between the deduced amino acid sequence of the *jadR*$_2$ product and the sequences of some comparably sized proteins believed to function as repressors, together with the enhanced production of jadomycin B when *jadR*$_2$ was disrupted, point to a role for this gene in the mechanism imposing rigorous environmental control of jadomycin B production.

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                                        Helix  Turn  Helix
                                      *********///**********
MtrR 1  mrktktealkTkehlmlaAletFyrkGiartslneIaqaagVtrgalywHFknKedlfda 60
EnvR 1 makrtkaealkTrqelietAiaqFaqhGvskttlndIadaanVtrgaiywHFenKtqlfne 61
AcrR 1 marktkqeaqeTrqhildvAlrlFsqqGvsstslgeIakaagVtrgaiywHFkdKsdlfse 61
TetC 3 nknhqqenfksTyqslvnsArilFvekGyqavsideIsgkalVtkgafyhHFknKkqllsa 63
TcmR 18 pglrqrklrrTrdqlireAlelFlaqGyehttveqIaeaveVhprtffrHFasKeevalt 77
JadR2 1  mtkqeratrTrdaliksAareFdehGyalaklsaIssgagVspgalhfHFenKvaaave 59
             ++ + + ++ ++ +  +   ++ ++   +   +  ++  ++++    ++    + +
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FIG. 3. Alignment of JadR$_2$ with the N-terminal regions of EnvR, MtrR, AcrR, TetC, and TcmR by using PILEUP (6). Amino acids common to all sequences are capitalized. Amino acids common to the JadR$_2$ sequence and at least one other sequence are identified by + below the alignments. The region with the predicted helix-turn-helix motif is indicated above the alignments.

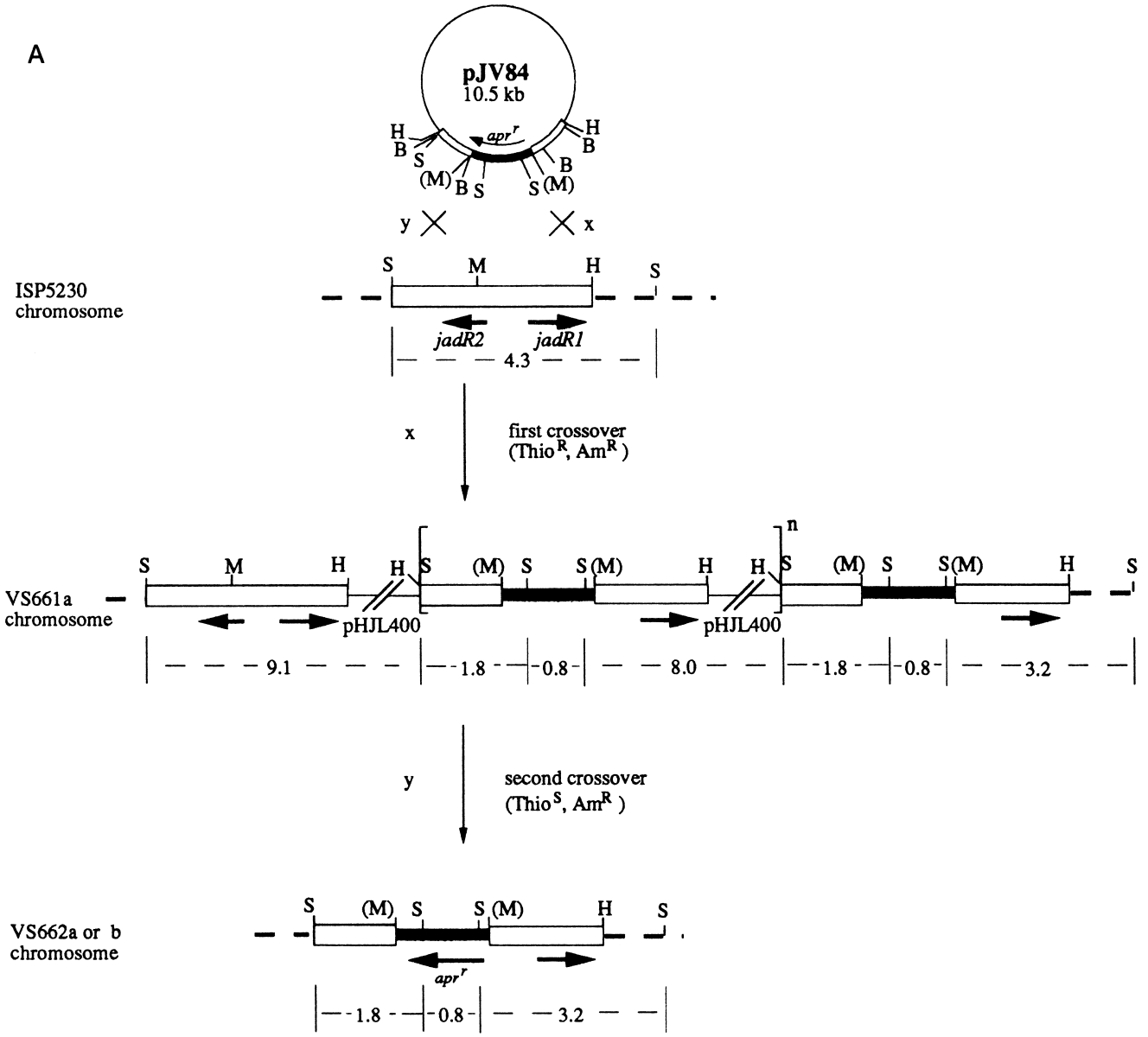

FIG. 4. (A) Disruption and replacement of $jadR_2$ by integration of pJV84. Wild-type *S. venezuelae* was transformed with the plasmid, and Thio^r Am^r colonies generated by a single crossover between homologous DNA in the plasmid and chromosome were selected. Progeny in which a second crossover had occurred were selected by their Thio^s Am^r phenotype. Filled bars represent the apramycin resistance gene (*apr^r*), open bars represent $jadR_2$ and flanking regions cloned from *S. venezuelae* DNA, a single continuous line represents vector (pHJL400) DNA, and divergent thick arrows indicate the locations and orientations of $jadR_1$ and $jadR_2$. Abbreviations: B, *Bam*HI; H, *Hin*dIII; M, *Mlu*I; S, *Sac*I. The *Mlu*I site lost during construction of pJV84 is in parentheses. (B) Southern hybridization of genomic and pJV84 DNAs digested with *Sac*I and probed with pJV84. Lanes: 1, ISP5230; 2, VS661a; 3, VS661b; 4, VS662a; 5, VS662b. Numbers on the right are sizes in kilobases.

Of the gene products resembling $JadR_2$ in the N-terminal regions of their amino acid sequences, MtrR from *Neisseria gonorrhoeae* and EnvR and AcrR from *E. coli* control tandemly linked genes that mediate resistance to structurally diverse antimicrobial agents by an efflux mechanism (28). Genetic and physiological analyses of the *mtr* system have demonstrated that mutations inactivating *mtrR* enhance resistance (11, 30); the repressor gene is located upstream from, and is oriented

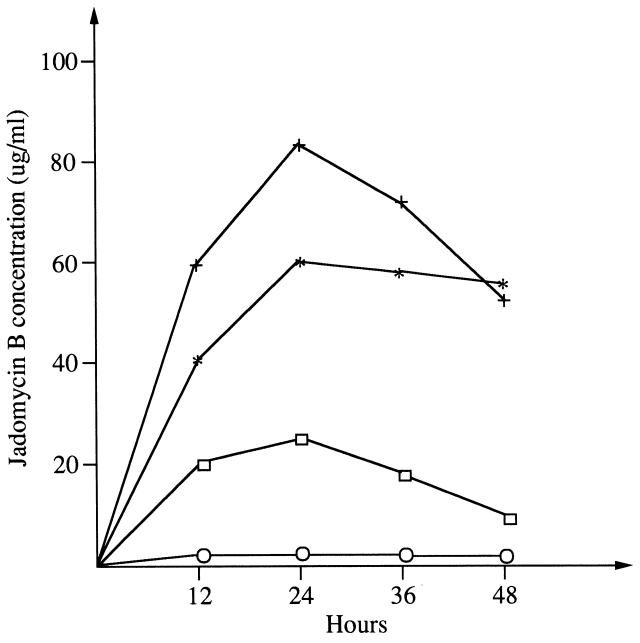

FIG. 5. Effect of ethanol treatment on the production of jadomycin B by *S. venezuelae* ISP5230 and VS662a. Cultures were grown in galactose-isoleucine medium alone or supplemented with ethanol (6%, vol/vol). Symbols: ○, ISP5230, untreated; ∗, ISP5230 treated with ethanol; □, VS662a, untreated; +, VS662a treated with ethanol.

oppositely to, the *mtrCDE* cluster of resistance genes (17). TetC in *E. coli* and TcmR in *S. glaucescens* also regulate genes conferring antibiotic resistance, probably by repression of an efflux mechanism (16, 34); as in the *mtr* cluster of *N. gonorrhoeae*, the repressor and resistance genes are divergently transcribed. $jadR_2$ resembles the repressor gene in these systems not only in the deduced amino acid sequences of the gene products but also in the presence upstream of a series of oppositely oriented ORFs. However, a comparison of the deduced amino acid sequences of the ORFs upstream of $jadR_2$ with the GenBank database has shown no similarities to the products of known antibiotic resistance genes. Whether the tolerance of *S. venezuelae* to jadomycin B is mediated by an efflux system of the type postulated for tetracenomycin C (16) is not known at present, since no genes conferring antibiotic resistance have yet been identified.

Since disruption of $jadR_2$ did not increase the level of jadomycin B production in untreated cultures to that in ethanol-treated cultures of the wild-type strain, and since the disrupted mutants retained the response to ethanol treatment, the failure of the wild type to produce jadomycin B under nutritional restraint alone cannot be attributed solely to repression by $JadR_2$. Ethanol treatment may activate the biosynthesis of jadomycin B in several ways, some of which are mediated directly by $jadR_2$ and others of which are mediated by modulating the derepression response.

The observation that jadomycin production in VS661 strains, in which integration of pJV84 by a single crossover should have retained $jadR_2$ intact in the chromosome, was not appreciably different from that in VS662 mutants, in which $jadR_2$ has been replaced by a disrupted form of the gene, was unexpected. The possibility that the similar phenotypes might be due to the high rate at which the intact copy of pJV84 was excised from the chromosome of VS661, resulting in a predominance of the V662 genome in jadomycin production cultures grown from VS661 spores, was excluded when addition to the culture medium of thiostrepton, which would select against the replacement strain, failed to decrease the amount of jadomycin produced. At present the results of vector integration and gene replacement are most readily explained by the participation of both positively and negatively acting genes in the regulation of jadomycin biosynthesis. The plausible candidates are $jadR_1$ and $jadR_2$, respectively. Because the disruption vector contained an intact copy of $jadR_1$ as well as the disrupted $jadR_2$, VS661 strains would carry an excess of $jadR_1$ relative to $jadR_2$. The excess would be increased by the multiple tandem integration of pJV84 that probably occurs in some VS661 strains, and this imbalance in the copy number of the two regulatory genes would likely offset repressive effects due to the presence of an intact copy of $jadR_2$ in VS661 strains.

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